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Postnatal evolution of the rat pineal gland: light microscopy

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INTRODUCTION

During postnatal life there is an important transformation of the albino rat pineal gland. Electron microscopy shows that the pineal gland of the neonatal rat contains only undifferentiated cells (Karasek, 1974; Calvo & Boya, 1983), the differentiation of cell types taking place during postnatal life (Karasek, 1974; Steinberg *et al.* 1981; Calvo & Boya, 1983). Biochemical studies also indicate that the appearance and development of biogenic amines and related enzymes take place after birth (Ellison, Weller & Klein, 1972; Klein, Namboodiri & Auerbach, 1981).

The postnatal transformation of the rat pineal gland has been studied mostly from a quantitative point of view using light microscopy. The quantitative modifications described include mitosis (Quay & Levine, 1957; Wallace, Altman & Das, 1969), increase in pineal volume (Izawa, 1925; Quay & Levine, 1957; Wallace *et al.* 1969; Blumfield & Tapp, 1970), and increase in pinealocyte size (Blumfield & Tapp, 1970). Although the results of these authors agree as to the general pattern of development, there are differences as to timing, intensity of growth, and other features.

The postnatal cytological changes of parenchymal cells are considerably less known. Only Tapp & Blumfield (1970) describe some of these changes, mostly the differentiation of pineal cell types. These authors describe three types of cell (I, II and III) which appear at two (I and II) and four weeks (III) respectively. Finally, Kappers (1960) also provides certain findings mostly on changes in shape and location of the rat pineal gland in the early postnatal period.

The present study describes the changes observed using light microscopy in the rat pineal gland from birth until ten months of age.

MATERIALS AND METHODS

For this study, albino Wistar rats of both sexes were kept under standard conditions of light and feeding. The rats were anaesthetised with ether and killed by decapitation at the following ages: 1, 3, 5, 7, 10, 15, 20, 25, 30, 45, 60 and 75 days; thereafter, animals were killed at each month from 3 to 10 months. At each age, four rats of each sex were taken. The whole brain including the pineal gland was fixed by immersion in Bouin's fluid, embedded in paraffin and serially sectioned at a thickness of 7 μm. The blocks were oriented in order to obtain sagittal and frontal sections of the gland. The staining techniques used were haematoxylin and eosin, Mallory's phosphotungstic haematoxylin and Gordon & Sweet's silver technique for reticular fibres.

In order to quantify the postnatal transformation of the gland, two parameters were determined in rats from 1 to 180 days of age (Fig. 1). The mid-sagittal section

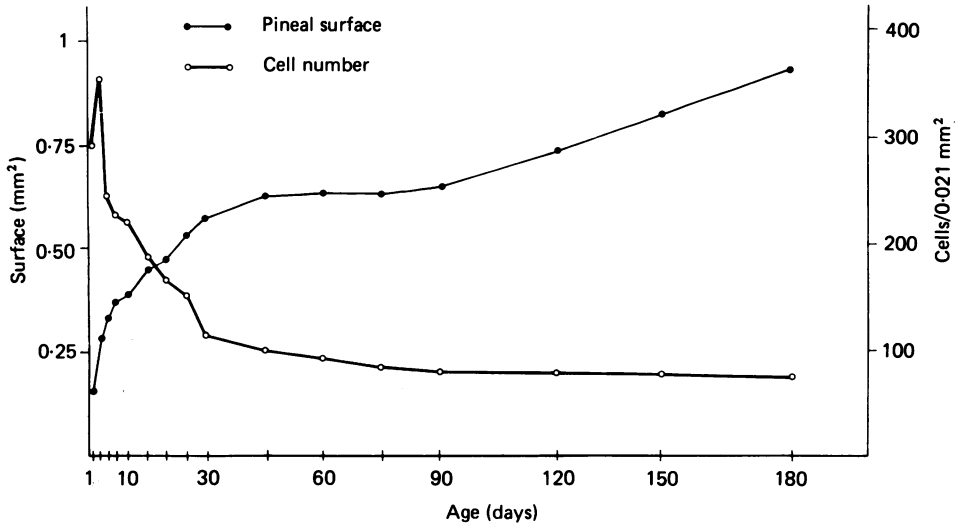

Fig. 1. Graph showing variations of mid-sagittal section surface area and number of parenchymal cells per unit area from one day until six months after birth. The ordinate axis shows the mean values.

of the gland was projected on paper and its surface area was measured with a planimeter. Also, the cell population was determined using plastic sections 0·5 μm thick stained with toluidine blue. The number of parenchymal cells was counted in eight areas each 0·021 mm² (four in the central region and four in the periphery) for all the specimens. After 60 to 75 days large connective tissue spaces were avoided in the areas counted.

RESULTS

Day 1

The pineal gland displayed an ovoid shape (Fig. 2) and was joined by a large base to the roof of the third ventricle. The pineal recess remained as a short and narrow proximal cleft (Fig. 2). The parenchyma was formed by large masses of small cells, many of them in mitosis (Fig. 3). The connective tissue spaces were few and small, tending to be located in the periphery, mostly in the distal end of the gland. In the centre there were large masses of cells with very few connective tissue spaces.

Fig. 2. One day female. Mid-sagittal section of the pineal gland. The pineal axis is almost vertical, perpendicular to the skull (asterisk). The pineal stalk is not yet formed. Haematoxylin and eosin. × 30.

Fig. 3. One day male. Pineal parenchyma formed by masses of apparently undifferentiated cells among which mitoses (encircled) are observed. Haematoxylin and eosin. × 500.

Fig. 4. Three days female. Small cells with dense nuclei forming cords among which large clear cells are found. Haematoxylin and eosin. × 200.

Fig. 5. Ten days male. Mid-sagittal section of the pineal gland showing formation of the pineal stalk. The pineal axis has shifted dorsally. Compare with Fig. 2. Asterisk, skull. Haematoxylin and eosin. × 30.

Fig. 6. Ten days male. Beginning of pineal cell type differentiation. Arrow, type II pinealocyte; *m*, mitosis. Haematoxylin and eosin. × 875.

Fig. 7(*a–b*), Ten days female. Degenerated cells (arrows) in the pineal parenchyma. (*a*) in subcapsular location; (*b*) in the vicinity of a connective tissue space. Haematoxylin and eosin. × 925.


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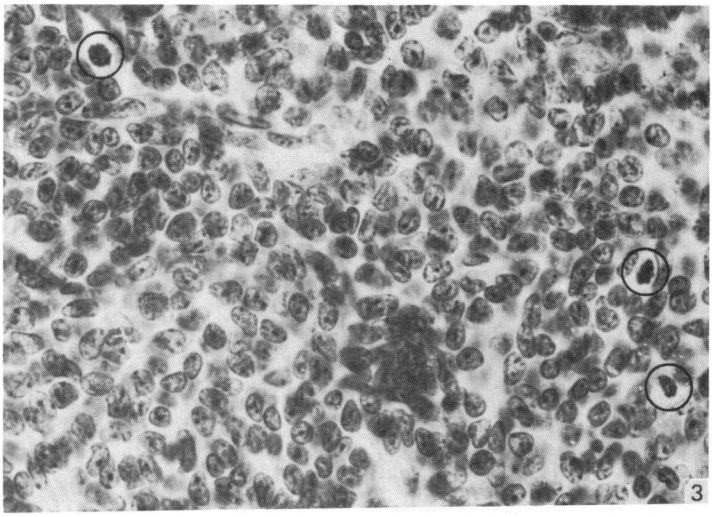
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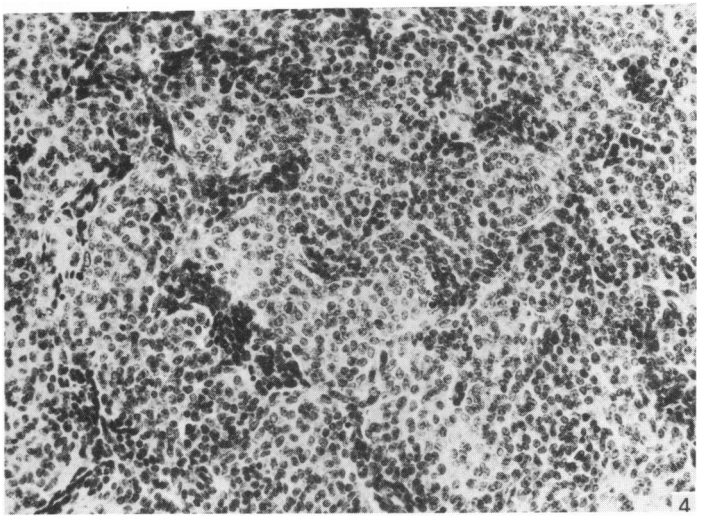
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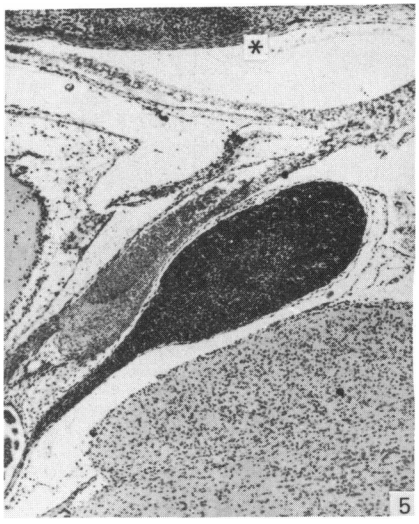
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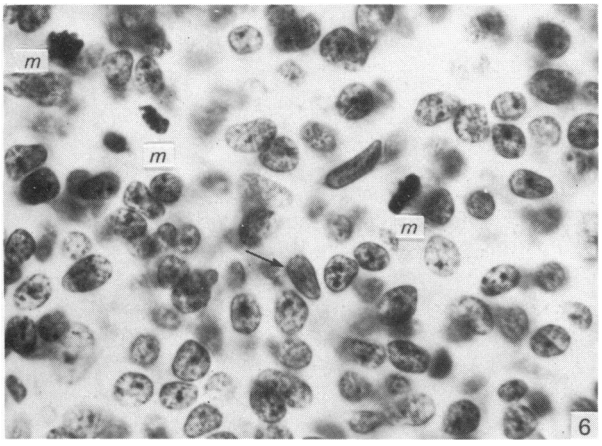
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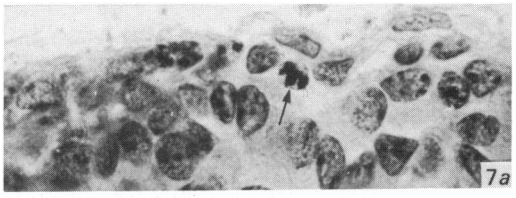
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Day 3

Surface area of the pineal section increased compared with that at Day 1 after birth (Fig. 1). The parenchyma displayed great cellularity, reaching the maximum peak in the number of cells per unit area at this stage (Fig. 1). At low magnifications, the pineal parenchyma showed small undifferentiated cells with dense nuclei forming ramifying cellular cords, the distribution of the cells being reminiscent of connective tissue (Fig. 4). Mitoses were frequently found here. Among the cords there were large masses of clearer cells with much cytoplasm (Fig. 4).

Days 5 *to* 10

The size of the gland continued to increase (Fig. 1). At this stage, the roof of the third ventricle no longer occupied the rather superficial location it had presented until now. Thus, the gland became elongated and was attached to the third ventricle by a long narrow stalk (Fig. 5). The axis of the gland was no longer vertical, and progressively adopted an oblique orientation (Fig. 5). The most marked change in position and shape of the gland took place between Days 7 and 10 after birth.

The parenchymal cells began to increase in size, with a decrease in the number of cells per unit area of section (Fig. 1). After ten days, differences in the nuclei of parenchymal cells (Fig. 6) began to define the two types of pinealocyte found in adults described below. However, many parenchymal cells still displayed the immature appearance of the nucleus found in younger animals.

Degenerated cells were constantly found in the pineal parenchyma during the first stages after birth (Fig. 7*a*, *b*) and were more frequent between Days 5 and 15. They contained a small pyknotic nucleus with dense granules of chromatin. Sometimes groups of two or three degenerate cells were found under the capsule (Fig. 7*a*) or near a connective tissue space (Fig. 7*b*).

Days 15 *to* 20

The pineal parenchyma already showed the cord-like appearance typical of this gland. In the periphery, there were groups of nuclei radially arranged around a central space apparently occupied by cytoplasm, forming 'pseudo-rosettes'. There was a generalised differentiation of cells. Cords of immature cells were no longer found.

Fig. 8. Fifty days male. Pineal stroma. Numerous thin connective tissue septa formed mostly by reticular fibres. Gordon and Sweet's silver technique. × 90.

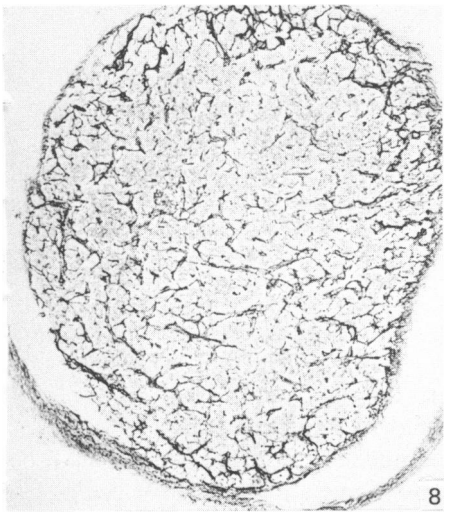
Fig. 9. Sixty days male. Differences in nuclear morphology between pinealocytes I and II. Arrowheads, Type II pinealocytes. Haematoxylin and eosin. × 825.

Fig. 10. Sixty days female. Arrangement of pinealocytes forming 'pseudo-rosettes.' Haematoxylin and eosin. × 950.

Fig. 11. Six months female. Mid-sagittal section of the pineal gland showing increase in gland volume (compare with Figs. 2 and 5). Haematoxylin and eosin. × 30.

Fig. 12. Seven months male. Increase in size of connective tissue septa, mostly in the periphery (compare with Fig. 8). Gordon and Sweet's silver technique. × 90.

Fig. 13. Eight months female. Intrapineal lymphoid nodule. Circles, 'pseudo-rosettes.' Haematoxylin and eosin. × 200.



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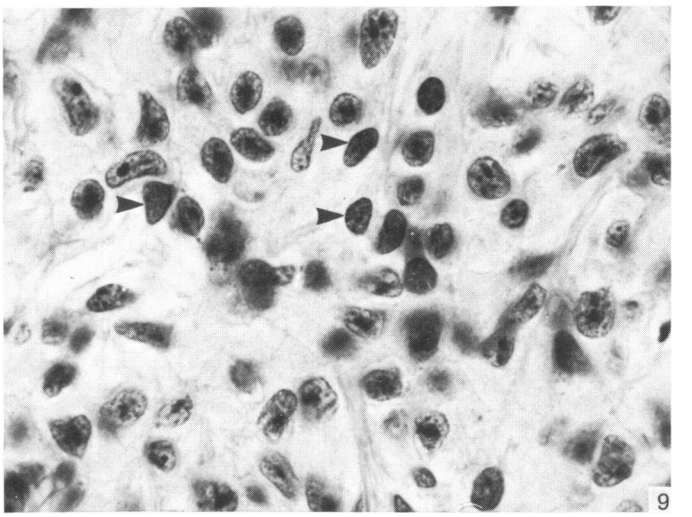

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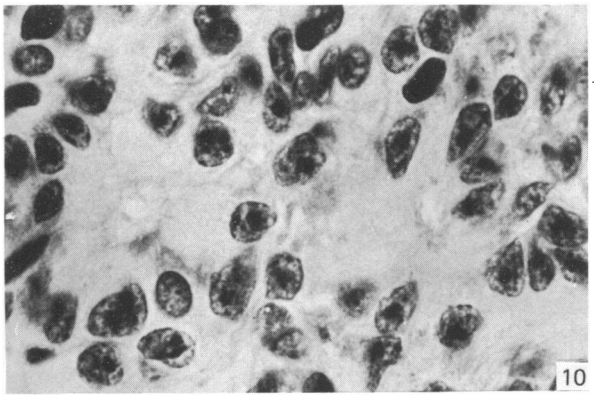

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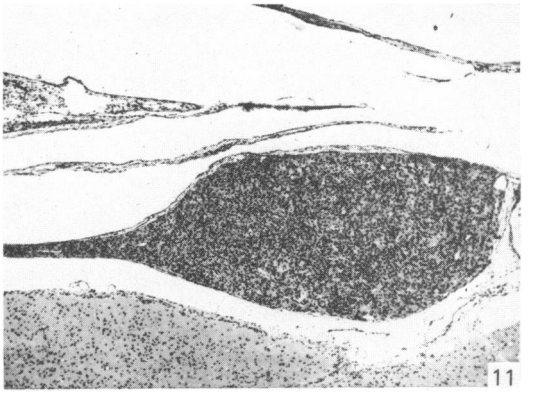

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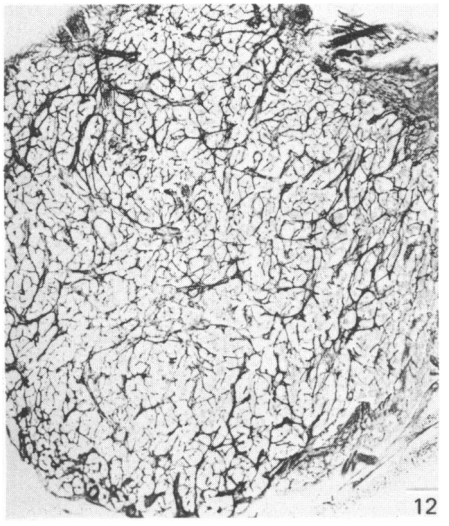

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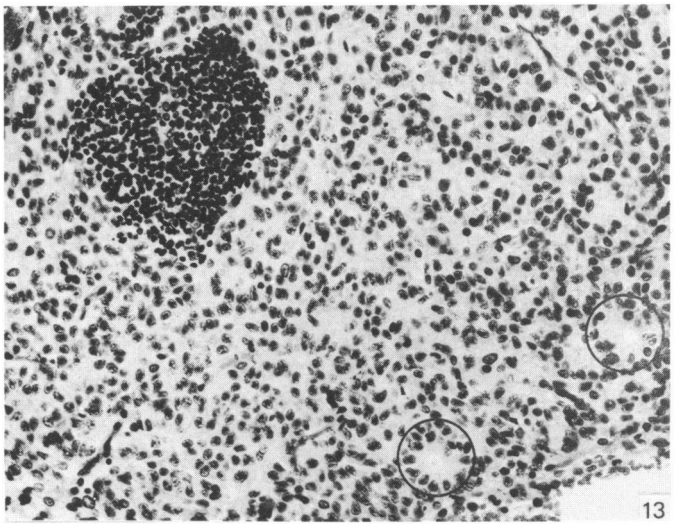

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Days 25 *to* 30

At this stage, the gland had reached a considerable size. Its mid-sagittal section was three to three and a half times greater than that found at Day 1 (Fig. 1). Pinealocyte hypertrophy was now such that the number of cells per unit area decreased to 35–40 % of the number found during the first days after birth (Fig. 1). Mitoses were now very rare.

Days 45 *to* 60

Pinealocyte hypertrophy continued although growth rate was slower than in previous stages (Fig. 1). Silver staining techniques showed abundant connective tissue spaces with more fibres than in earlier stages. The wider connective tissue septa were located in the periphery of the gland, mostly in its distal portion (Fig. 8).

Two cell types were easily identified by their nuclear characteristics. Type I pinealocytes showed large, round nuclei with pale chromatin and prominent nucleoli. These formed 85–90 % of parenchymal cells (Fig. 9). Type II pinealocytes were fewer (10–15 % of cells) and they presented smaller ovoid nuclei containing a more dense and homogeneous chromatin (Fig. 9). The nuclei of endothelial and connective tissue cells were usually smaller and denser than Type II cell nuclei. The 'pseudo-rosettes' observed earlier were more numerous and better defined than in previous stages. Inside them, there was a central space limited by the apical cytoplasm of pinealocytes (Fig. 10).

Adult stage

From 75 days onwards the changes were much more gradual. Pineal size increased slowly from 75 days until 6 months when the area of the mid-saggittal section was five times greater than at the first day after birth (Figs. 1, 11).

In the adult rat, the shape of the pineal gland was comparable to a chestnut with a flattened dorsoventral axis. In its longitudinal (fronto-occipital) axis, the gland measured between 1500 and 1600 μm while transversely it measured 1300 to 1500 μm; dorsoventrally the maximum measurement was 750 to 800 μm. The gland was attached to the roof of the third ventricle between the anterior and posterior commissures by a long (2700 to 3000 μm) thin stalk which arose from its proximal end.

In the periphery of the gland, pinealocytes with large amounts of cytoplasm tended to form rounded islets and cords separated by thick septa. Numerous 'pseudo-rosettes' were found here (Fig. 13). In the centre of the gland, pinealocytes adopted a more compact arrangement. Some 'pseudo-rosettes' were also found here.

Cell counts indicated that pinealocyte hypertrophy continued slowly from 75 days until 6 months (Fig. 1). Few changes were found in pinealocyte structure, except for the appearance and progressive development of nucleoli and nuclear envelope infoldings in Type I pinealocytes. With age, there was also a progressive increase in the number of connective tissue fibres (Fig. 12).

Two special components were located in the pineal stroma. The first of them was striated muscle, which was usually located in the wide connective tissue septa near the capsule. It consisted of thin myocytes usually forming bundles of a few muscle fibres. These muscle fibres appeared only rarely, always in adult rats in the present investigation.

The second component consisted of lymphoid cells (Fig. 13). They were first observed at Day 45, after which they were constantly found in the pineal gland. There

were considerable differences in their number between different animals, although the total amount was usually not large. In most rats, lymphoid cells infiltrated some of the connective tissue septa and the neighbouring parenchyma. Occasionally, small nodules were present in the interior of the gland (Fig. 13). Less often, large nodules were located in the pineal capsule, usually extending into the interior of the gland. These capsular nodules were usually associated with diffuse lymphoid infiltrates in the capsular connective tissue.

DISCUSSION

Figure 1 shows the important changes in the rat pineal gland during postnatal life. The surface area of the mid-sagittal section of the gland increases sixfold during the first 180 days after birth. This growth has two phases: there is a rapid increase in size until Day 45, and a slower growth from Day 45 until six months. Blumfield & Tapp (1970) and Walker, McMahon & Pivorun (1978), who have studied variations in pineal weight, also find these two phases. Quay & Levine (1957), and Wallace *et al.* (1969) have also studied the increase in area of a mid-sagittal section of the pineal up to three to four months. The values obtained by the present study are slightly greater than theirs, although the shapes of both curves are similar. Wallace *et al.* (1969) describe a decrease in the surface area of section from 60 to 120 days, but the present results show an increase in surface area until at least six months of age. Variations in pineal gland weight also seem to follow a similar course (Izawa, 1925; Blumfield & Tapp, 1970; Walker *et al.* 1978).

The marked decrease in the number of parenchymal cell nuclei after Day 3 indicates a hypertrophy of these cells. Pyknotic nuclei are seen in the first two postnatal weeks but their total number is nevertheless rather small. Thus, although cell deaths may cause some of the decrease in cell number, mainly between Days 5 and 10, their contribution to the total decrease is probably not significant. Various values for pinealocyte hypertrophy in the postnatal rat have been obtained. According to Izawa (1925), the number of nuclei decreases by a third between 20 days and 5 months. According to Wallace *et al.* (1969) and Blumfield & Tapp (1970), there is a 50 % decrease from birth to maturity. According to Quay & Levine (1957), at 16 weeks the number of nuclei is approximately 35 % of that after birth. The present results, however, indicate that at six months the number of nuclei per unit area is only 20 % of the number found on the first postnatal day. The difference could be due to the technique employed in this study (plastic embedding with sections 0·5 μm thick), which permits a clearer identification of the cell types and produces less shrinkage during processing of samples.

The changes in the number of cells per unit area would thus explain the form of the curve for area of the mid-sagittal section. The increase in number of nuclei per unit area from Day 1 to Day 3 corresponds to the stage of maximum cell proliferation, already described by other authors (Quay & Levine, 1957; Wallace *et al.* 1969). After three days, although proliferation continues, hypertrophy of parenchymal cells begins. This confirms earlier results of Calvo & Boya (1983) using the electron microscope. From Day 3 until Day 20, in which mitoses are difficult to find, the progressive hypertrophy and differentiation of pinealoblasts account for both decrease in number of nuclei and gland growth. Thus, after the first week, the contribution of cell proliferation to gland growth is minimum. Young pinealocytes present at Day 20 continue to enlarge until 45–60 days after birth when their structure is similar to that of the adult cell (Calvo & Boya, 1983). At this stage, the rapid

decrease in the number of nuclei and increase in mid-sagittal section surface area ends. After Day 60, hypertrophy continues at a very slow rate.

There are also important changes in shape and position of the pineal gland during postnatal life. The gland displays an ovoid shape in the neonatal rat. Postnatal growth is considerable along the sagittal and transverse or horizontal axes (two- to threefold) but is less evident along the vertical axis (one and a half times), thus accounting for the chestnut-like flattened shape of the gland in the later stages. Modifications in the vertical alignment of the pineal axis to a very oblique, almost horizontal, axis account for the change in gland position. This inclination of the pineal gland has been cited previously by Kappers (1960). The present results indicate that these changes take place mostly between Days 7 and 10 after birth.

After Day 10, differences in nuclear structure define two types of pinealocyte as seen by the light microscope. Tapp & Blumfield (1970), responsible for the only other study of pineal gland cell differentiation using the light microscope, identify two cell types in two weeks old rats, and a third type at four weeks. The first two types probably correspond to the Type I cell described in the present study, and their third type to the Type II pinealocyte. Using the light microscope, no differences have been found in the present investigation which justify a subdivision of the Type I pinealocyte into two varieties as described by Tapp & Blumfield (1970). Finally, these authors also mention the appearance in rats at two weeks of age of 'pseudo-alveoli' which undoubtedly correspond to the 'pseudo-rosettes' described here, a frequent characteristic cell arrangement in the rat pineal gland which has not yet been sufficiently studied.

The presence of lymphocytes in the rat pineal gland has been described previously only by Uede *et al.* (1981). These authors find massive clusters of lymphoid cells in the pineal capsule of rats 90–120 days old. Immunohistochemical techniques identify these cells as T lymphocytes. Although the number of lymphocytes varies among animals, the present results confirm the incidence of these cells in the adult rat. In the material used here, nodular or disperse intrapineal lymphoid infiltrates are more frequent.

Several authors (Quay, 1959; Kappers, 1960; Tapp & Blumfield, 1970) describe striated muscle fibres as a very rare component of the rat pineal gland. Diehl (1978), however, finds them in one third of animals investigated using serial sections stained with haematoxylin and eosin. Our results in sections stained with phosphotungstic haematoxylin show that these fibres are a rather infrequent finding in the rat pineal gland.

SUMMARY

The postnatal development and morphology of the adult albino rat pineal gland was studied from one day up to ten months of age. During postnatal life there was a marked increase in gland and pinealocyte volume, more intense during the first 45 days. After ten days, the differences in nuclear morphology of parenchymal cells showed two different types of pinealocyte. The characteristic adult arrangement of pinealocytes in cords and pseudo-rosettes was observed after 15–20 days. After 75 days there was a progressive increase in the number of connective tissue fibres.

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